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Invited article

Classification, Molecular Phylogeny, Divergence Time, and Morphological Evolution of Pteridophytes with Notes on Heterospory and Monophyletic and Paraphyletic Groups

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Pteridophytes are free-sporing vascular land plants that evolutionarily link bryophytes and seed plants. Conventional, group (taxon)-based hierarchic classifications of pteridophytes using phenetic characters are briefly reviewed. Review is also made for recent tree-based cladistic analyses and molecular phylogenetic analyses with increasingly large data sets of multiple genes (compared to single genes in previous studies) and increasingly large numbers of species representing major groups of pteridophytes (compared to particular groups in previous studies), and it is extended to most recent analyses of estimating divergence times of pteridophytes. These classifications, phylogenetics, and divergence time estimates have improved our understanding of the diversity and historical structure of pteridophytes. Heterospory is noted with reference to its origins, endospory, fertilization, and dispersal. Finally, monophyletic and paraphyletic groups recently proposed or re-recognized are briefly described.

Key words: classification, divergence time estimate, ferns, heterospory, molecular phylogeny, pteridophytes.

Morphological Classifications

Pteridophytes, like seed plants (gymnosperms and angiosperms), are vascular land plants and also are similar to nonvascular bryophytes in the free-sporing reproduction. Evolutionarily they followed bryophytes and preceded seed plants. Thus, free-sporing vascular plants or pteridophytes in a broad sense have a long (420 million years) evolutionary history, and on the other hand, like angiosperms, exhibit a recent diversification, resulting in a total of about 12,000 species, which may be primitive or advanced. The species were classified in many different classification systems based on morphological characters. Some of major classifications put forward in the 20th century are briefly noted here (Tables 1, 2).

Engler & Prantl (1902) classified pteridophytes into four classes, Filicales, Sphenophyllales, Equisetales and Lycopodiales, and subdivided the

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TABLE 1. Classifications of major pteridophyte groups proposed by some authors, based on comparative morphology. Numbers indicate grouping in each classification and do not correspond among classifications.

Engler & Prantl (1902)	Verdoorn (1938)	Tagawa & Iwatsuki (1972)	Pichi Sermolli (1977)	Ching (1978)	Tryon & Tryon (1982)	Kramer & Green (1990)
1. Lycopodiales	1. Lycopodiinae	1. Lycopsida	1. Lycophytina	1. Lycophytina	1. Lycoodiopsida	1. Lycopodiatae
1-1. Ligulatae						
1-1-1. Selaginellineae	1-1. Selaginellales	1-1. Selaginellales	1-1. Selaginellales	1-1. Selaginellales	1-1. Selaginellales	1-1. Selaginellales
1-1-2. Isoetineae	1-2. Isoetales	1-2. Isoetales	1-2. Isoetales	1-2. Lycopodiales	1-2. Isoetales	1-2. Isoetales
1-2. Eligulatae						
1-2-1. Lycopodineae	1-3. Lycopodiales	1-3. Lycopodiales	1-3. Lycopodiales	2. Isoephytina	1-3. Lycopodiales	1-3. Lycopodiales
1-2-2. Psilotineae	2. Psilophytinae	2. Psilopsida	2. Psiophytina	3. Psiophytina		2. Psilotatae
2. Equiestales	3. Articulatae	3. Equisetopsida	3. Sphenophytina	4. Sphenophytina	2. Equisetopsida	3. Equisetatae
3. Sphenophyllales †						
4. Filicales	4. Filicinae	4. Pteropsida	4. Filicophytina	5. Filicophytina	3. Filicopsida	4. Filicatae (incl. Psilotaceae)

† Extinct group included in Equisetopsida in other classifications.

TABLE 2. Classifications of major fern groups proposed by some authors, based on comparative morphology. Numbers indicate grouping in each classification and do not correspond among classifications.

Christensen (1938)	Copeland (1947)	Tagawa & Iwatsuki (1972)	Pichi Sermolli (1977)	Ching (1978)	Tryon & Tryon (1982)
1. Eusporangiatae				1. Eusporangiopsida	1. Polypodiidae
1-1. Ophioglossales	1. Ophioglossales	1. Ophioglossales	1. Ophioglossopsida	1-1. Ophioglossales	1-1. Ophioglossales
1-2. Marattiales	2. Marattiales	2. Marattiales	2. Marattiopsida	1-2. Marattiales	1-2. Marattiales
2. Leptosporangiatae	3. Filicales	3. Filicales	3. Filicopsida	2. Protoleptosporangiopsida	1-3. Polypodiales
2-1. Filicales			3-1. Osmundidae	3. Leptosporangiopsida	1-3-1. Polypodiineae
			3-2. Plagiogyriidae	3-1. Polypodiales	
			3-3. Gleicheniidae		
			3-4. Schizaeidae		
			3-5. Hymenophyllidae		
2-2. Salviniales		4. Salviniales	3-6. Salviniidae	3-2. Salviniales	1-3-2. Salviniineae
		5. Marsileales	3-7. Marsileidae	3-3. Marsileales	1-3-3. Marsileineae
					2. Psilotidae

class Filicales into three orders, Filicales leptosporangiatae (suborders Eufilicineae and Hydropteridineae), Marattiales and Ophioglossales, and the class Lycopodiales into two orders Lycopodiales eligulatae including suborder Psilotineae and Lycopodiales ligulatae with suborders Selaginellineae and Isoetineae. Christensen's (1905, 1913-1934) Index Filicum and supplements I-III enumerated all fern species of the world described. In his systematic classification of ferns Christensen (1938) recognized two series (Filices Eusporangiatae and Filices Leptosporangiatae), two orders Ophioglossales and Marattiales in the former series and two orders Filicales and Salviniales in the latter, and in total 14 families including the large family Polypodiaceae with 15 subfamilies. Ching (1940) classified "Polypodiaceae" into 33 families and recognized five series in the polyphyletic "Polypodiaceae" in the context of phylogeny. Ching (1978) classified Chinese pteridophytes (division Pteridophyta) into five subdivisions: subdiv. Lycophytina comprising orders Lycopodiales and Selaginellales, three monotypic subdiv. Isoephytina, Sphenophytina and Psilophytina, and subdiv. Filicophytina comprising three classes (Eusporangiopsida [orders Ophioglossales and Marattiales], Protoleptosporangiopsida [Osmundales], and Leptosporangiopsida [Polypodiales or Filicales,

Marsileales, Salviniales]). In Genera Filicum Copeland (1947) recognized orders Ophioglossales (1 family), Marattiales (1 family), and Filicales (19 families including Marsileaceae and Salviniaceae). Holttum (1949) classified leptosporangiate ferns into 14 families including the large family Dennstaedtiaceae with 11 subfamilies and proposed three phylogenetic lineages, of which one is terminated by Dennstaedtiaceae. Tagawa & Iwatsuki (1972) adopted the conventional classification of pteridophytes into four classes Psiopsida, Lycopsida, Equisetopsida, and Pteropsida. They classified Pteropsida into orders Ophioglossales, Marattiales, Filicales, Marsileales, and Salviniales. Tagawa & Iwatsuki (1972) recognized in total 34 families for pteridophytes of Thailand. Pichi Sermolli (1977) classified Pteridophyta into four subdivisions, Lycophytina, Sphenophytina, Psilophytina, and Filicophytina. The first three were monotypic each with single classes, while the last Filicophytina comprised three classes, Ophioglossopsida, Marattiopsida and Filicopsida. Pichi Sermolli (1977) assigned 58 of 64 families to Filicopsida, 3 to Lycopsida, 1 to Equisetopsida, and 2 to Psilotopsida. Kramer & Green (1990) compiled contributions to pteridophyte classification and presented a similar classification system of four classes Psilotatae (1 family), Lycopodiatae (3 families), Equisetatae (1 family), and Filicatae (33 families). Among families of Filicatae, affinities were suggested between Dipteridaceae and Cheiropleuriaceae; Vittariaceae and Pteridaceae; tree fern families; Lomariopsidaceae, Davalliaceae, Nephrolepidaceae, Oleandraceae and Dryopteridaceae; Polypodiaceae and Grammitidaceae; and Azollaceae and Salviniaceae. Tryon & Tryon (1982) divided Division Pteridophyta into three classes Filicopsida, Equisetopsida (with a single order Equisetales) and Lycopodiopsida, and subdivided Filicopsida into two subclasses Polypodiidae and Psilotidae. Tryon & Tryon (1982) further divided Polypodiidae into orders Ophioglossales, Marattiales, and Polypodiales (suborders Polypodiineae, Marsileineae, and Salviniineae), and divided Lycopodiopsida into Lycopodiales, Selaginellales and Isoetales. Tryon & Tryon (1982) included Psilotaceae in Filicopsida (ferns) based on Bierhorst's (1977 and references cited therein) morphological and anatomical results and spore wall characters.

In short, the classifications based on phenetic characters usually recognized four major groups of living pteridophytes, among which ferns were divided into Marattiales, Ophioglossales and Filicales, although certain groups (*e.g.,* Psilotaceae and aquatic ferns) were assigned to different groups of higher ranks or treated at different ranks (Tables 1, 2). Characters that are informative throughout pteridophytes are not many. Those classifications with hierarchic ranks are generally taxon-based and have usually not been given statistically analyzed interrelationships of families.

Molecular Phylogenies

Achievement of molecular phylogeny, which is displayed as a phylogenetic tree, succeeded long contributions of systematics, classifications, and floras based on phenetic or morphological characters, as noted above. Molecular analyses with large data sets dealing with all or most pteridophyte groups have been explosive since the middle 1990s. One year later than Chase *et al.*'s (1993) epoch-making study on angiosperm phylogeny using a large data set (ca. 500 operational taxonomic units), Hasebe *et al.* (1994) presented a molecular phylogeny of leptosporangiate ferns deduced from *rbcL* sequences of 58 species representing almost all families recognized in the then classifications (Kramer & Green 1990). The number of families they dealt with was larger than that of any previous molecular analyses, although the number of species per family was few. Hasebe *et al.*'s (1994) pioneer work solved several of significant questions on pteridophyte phylogeny and accelerated research to solve them. One of their

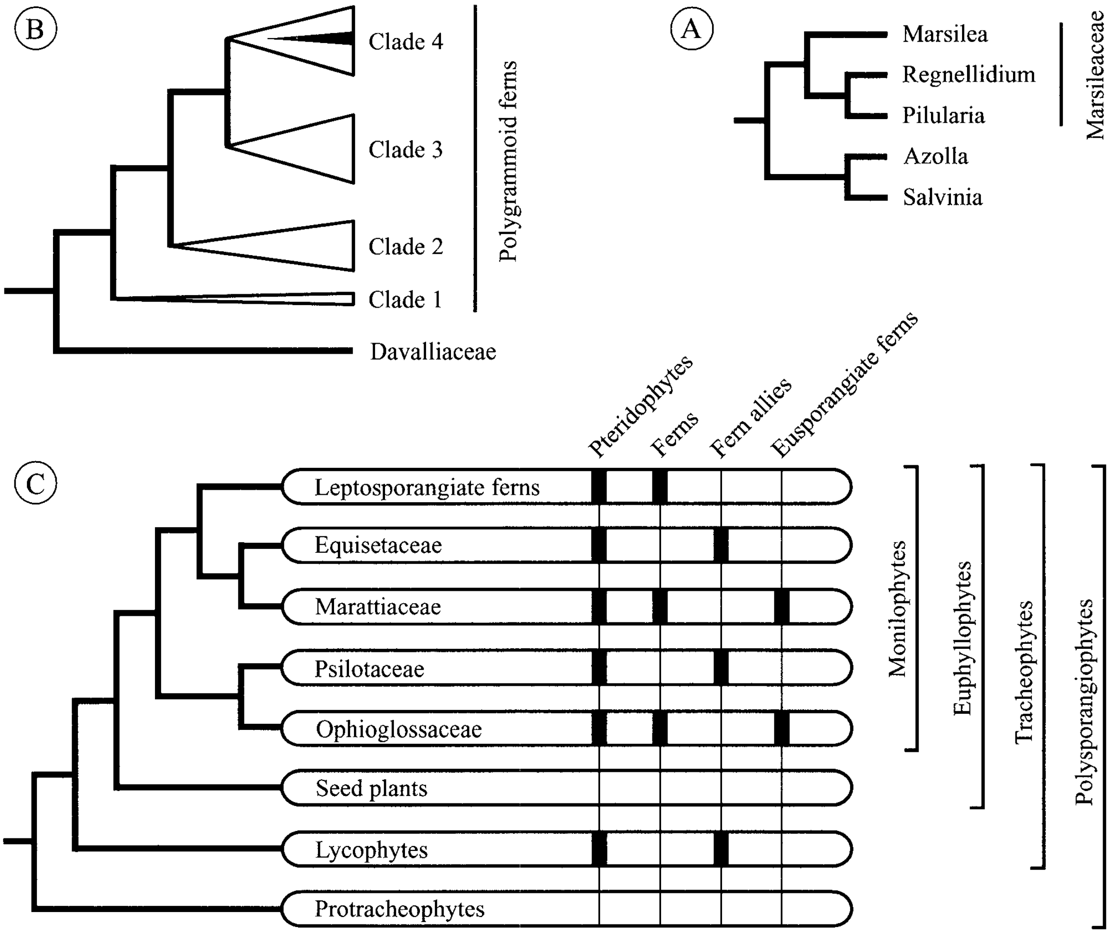

FIG. 1. Monophyletic and paraphyletic groups of pteridophytes. A. Monophyletic aquatic ferns. *Azolla* and *Salvinia* are assigned to Salviniaceae or each genus, to monotypic family. Tree is adapted from Pryer (1999). B. Monophyly of Polypodiaceae and Grammitidaceae (polygrammoids) and that of epiphytic polygrammoid ferns and Davalliaceae. Four clades of polygrammoid ferns are defined by Schneider *et al.* (2004b). Solid triangle in clade 4 indicates Grammitidaceae. C. Major groups of polysporangiophytes. Groups on the right side are monophyletic, and groups on the top are paraphyletic. Protracheophytes may be paraphyletic. Part of drawing is modified from Pryer *et al.* (2004b).

findings is that ferns that were considered primitive by morphological systematics diverge earlier than morphologically more advanced groups. For example, Osmundaceae are the basalmost in leptosporangiate ferns, and Hymenophyllaceae and a group of Gleicheniaceae, Dipteridaceae (also Cheiropleuriaceae) and Matoniaceae are the second and third basal. In comparison, Vittariaceae, Pteridaceae, Polypodiaceae, Davalliaceae, Dryopteridaceae, and some others are branched later. This order of branching is in good accordance with the polarity of trend of sporangia. The sporangium of Osmundaceae is massive and, along with Schizaeaceae, has a lateral or apical annulus. The sporangium of Hymenophyllaceae and Gleicheniaceae has an oblique annulus, while higher leptosporangiate ferns have small, flattened sporangia with vertical annuli. It is hypothesized that the sporangium morphology evolved from a massive to small capsule and from the distal to oblique and then to vertical annulus (Bower 1935).

The second of their findings is that tree fern families, Cyatheaceae, Dicksoniaceae and Metaxyaceae, along with Plagiogyriaceae, are monophyletic, although Plagiogyriaceae are not typically tree ferns. Traditionally, the two tree fern families

are considered not to be closely related by differences in dermal appendages and sori: Cyatheaceae are characterized by having scales and superficial sori, and Dicksoniaceae, by having hairs and marginal sori. Metaxyaceae, like Plagiogyriaceae, show a combination of hair appendages and superficial sori (soenosori). Recently, Hymenophyllopsidaceae, a small nontree fern family endemic to the Venezuelan Guayana, Guyana and Brazilian sandstone highlands (tepuis), were unraveled to have a close relationship with the tree fern group (Wolf *et al.* 1999). It supports monophyly of the tree fern and related nontree fern families. Hence, this tree-fern clade saw marked evolutionary changes in stem habit, dermal appendage, lamina histology, soral position, and indusium.

The third of Hasebe *et al.*'s findings is worthy of special mention. It is that the aquatic and heterosporous fern families, Azollaceae, Marsileaceae, and Salviniaceae, form a monophyletic clade (Fig. 1A). Aquatic life and heterospory are usually considered to be a curiously enough sharing, because the families are so distinct in the vegetative and reproductive characters as to be placed distantly or classified at higher ranks in traditional classifications, as noted above. Rothwell & Stockey (1994) discovered a fossil fern, *Hydropteris*, and interpreted that it has intermediate morphologies between Marsileaceae and Salviniaceae. Rothwell & Stockey (1994) recognized the order Hydropteridales comprising the fossil *Hydropteris* and the extant Azollaceae, Marsileaceae and Salviniaceae. Hasebe *et al.*'s (1994) molecular evidence is in good accordance with Rothwell & Stockey's (1994) treatment of the aquatic fern families. Monophyly of the aquatic ferns is also supported by a combined molecular and morphological analysis (Pryer 1999). The combination of aquatic life and heterospory will be discussed below.

The fourth of their findings is monophyly of Polypodiaceae, Grammitidaceae and Davalliaceae (Fig. 1B). Polypodiaceae and Grammitidaceae differ in a few characters. Polypodiaceae usually have reticulate venation and only scales as appendages, while Grammitidaceae have free venation and acicular hairs besides scales. Later, a multigene phylogenetic analysis with much larger data sets shows that Grammitidaceae are nested within Polypodiaceae and the two families are together coined "polygrammoid" ferns (Schneider *et al.* 2002, 2004b). Furthermore, this polygrammoid group and Davalliaceae differ in that the sori are marginal and indusiate in Davalliaceae and superficial and exindusiate in the polygrammoid group. Comparison with their successive sisters (*e.g., Oleandra, Nephrolepis, and Arthropteris*) indicates that the exindusiate sorus of the polygrammoid ferns is an apomorphic character state. By contrast, they share densely scaly, long-creeping, dorsiventral rhizomes (but the rhizomes are short and the leaves are radially arranged in many Grammitidaceae), a complex dictyostelic vascular system of the rhizome, and epiphytism. Davalliaceae and the polygrammoids, like Vittariaceae, are typical epiphytic fern families. The monophyly suggests the origin of the epiphytic families from a common ancestor of a certain life form.

Phylogenetic analyses using increasingly large data sets in the number of groups and the length of DNA sequences followed. Hasebe *et al.*'s (1995) analysis with more species and families than Hasebe *et al.* (1994) presented similar results. In an analysis with 35 species of ferns and fern allies (*i.e.,* lycophytes, *Equisetum*, and Psilotaceae) and multiple genes (three chloroplast genes and a nuclear gene), Pryer *et al.* (2001) found noteworthy relationships of lower pteridophytes. Tracheophytes diverge first into the microphyllous lycophytes and the euphyllous plants (Fig. 1C). This divergence is in full agreement with the one that had been proposed from the chloroplast gene order (Raubeson & Jansen 1992). The euphyllous plants diverge into seed plants and euphyllous pteridophytes called monilophytes. The monilophytes in turn comprise

eusporangiate and leptosporangiate ferns, Psilotaceae, and *Equisetum*. Psilotaceae form a clade with the eusporangiate Ophioglossaceae, while *Equisetum* forms another clade with the eusporangiate Marattiaceae though support is low.

Pryer *et al.* (2004b) extended their analysis for 62 taxa, using the same four genes, and constructed almost the same phylogenetic tree as that of Pryer *et al.* (2001), although there is a difference in the position of Gleicheniaceae, Matoniaceae and Dipteridaceae (including Cheiropleuriaceae). After the divergence of Osmundaceae, the rest of leptosporangiate ferns divides into Hymenophyllaceae and all others in Pryer *et al.*'s (2001) tree, whereas in Pryer *et al.*'s (2004b) tree a monophyletic group of Hymenophyllaceae along with Gleicheniaceae, Matoniaceae and Dipteridaceae diverges from the remaining of ferns. Matoniaceae differ from the sister-group Dipteridaceae in that the former have discrete indusiate sori and the latter have acrostichoid exindusiate sori (i.e. sporangia are scattered), although they share hairs (scales absent). The phylogeny that the exindusiate Gleicheniaceae are likely sister to the Matoniaceae-Dipteridaceae clade (Hasebe *et al.* 1995, Pryer *et al.* 2004b) supports that the extant and fossil Matoniaceae with peltate indusia were derived from exindusiate members of the family (Kato & Setoguchi 1999).

The above results show sharp conflict to the traditional classifications of pteridophytes based on comparative morphology, with special reference to lower groups. Previously, pteridophytes were usually classified into ferns (Filicopsida) and three classes of fern allies, i.e. Psiopsida, Lycopsida, and Equisetopsida (Fig. 1C). The Lycopsida is defined by the monosporangiate microphyllous leaves. Psiopsida is characterized by the absence of roots and typical leaves, while Equisetopsida is characterized by the strobilus comprising sporangiophores with inwardly oriented sporangia and the whorled sphenophylls. Filicopsida has megaphyllous leaves bearing aggregates of sporangia (called sori). Because those differences are equally large between the four groups, the pteridophytes were usually classified on the class level. Ophioglossaceae are morphologically more similar to other megaphyllous ferns than to Psilotaceae, although molecular evidence suggests monophyly. The most conspicuous difference between Ophioglossaceae and Psilotaceae is the presence or absence of the root, but it is uncertain whether the root was lost in Psilotaceae or it never appeared at the appearance of the family. It is the case with *Equisetum*. The largest difference from Marattiaceae is seen in the reproductive organ and phyllotaxis. It is hypothesized that the sporangiophorous strobilus and whorled leaves appeared in the early divergence of Equisetopsida. Thus, the molecular phylogeny suggests that remarkable morphological diversification may have occurred in the early evolution of the basal euphyllous pteridophytes.

Divergence Time

As molecular data have been accumulated for pteridophyte phylogeny, analyses with large data sets have been extending to estimating the divergence times of major groups of pteridophytes. In penalized likelyhood analyses of ferns and angiosperms, Schneider *et al.* (2004a) demonstrated that most polypodioid (in a broad sense) or higher leptosporangiate ferns diversified in the Cretaceous (100 Mya or later; Mya = million years ago) after angiosperm radiation. They further suggested that the fern diversification was an ecological opportunistic response to the diversification of angiosperms, as angiosperms came to dominate terrestrial ecosystems. Recently, an unconventional photoreceptor phytochrome 3 was discovered in a polypodioid fern, *Adiantum pedatum* (Kawai *et al.* 2003). Phytochrome 3 functions for red-light-induced phototropism and for red-light-induced chloroplast photorelocation, thereby conferring a distinct advantage under low-light canopy conditions. Schneider *et al.*

(2004a) argued that the photoreceptor was involved in the diversification of ferns in angiosperm-dominant dense forest ecospaces.

A similar divergence time estimate, together with molecular phylogeny, was undertaken for basal ferns (Pryer *et al.* 2004b). Using penalized likelihood analyses of molecular data and constraints from a reassessment of the fossil record, Pryer *et al.* (2004b) estimated that basalmost fern families diverged during the Carboniferous and Jurassic. A most basal fern clade of Ophioglossaceae and Psilotaceae appeared near the end of the Devonian (364 Mya) and the two families diverged in the Late Carboniferous, while that of Marattiaceae and Equisetaceae appeared in the very Early Carboniferous (359 Mya) and the families diverged shortly after (354 Mya). Osmundaceae, the basalmost leptosporangiate ferns, diverged in the middle Carboniferous (323 Mya). Exceptionally, Hymenophyllopsidaceae, a nontree-fern member of the lower tree fern group, diverged much later in the Tertiary. Two aquatic fern families, Azollaceae and Salviniaceae, diverged in the Cretaceous.

Among recent progress in the systematics and divergence time estimate of particular fern groups, most noteworthy is Yatabe *et al.*'s (1999) work on Osmundaceae. Traditionally, based on morphological data, Osmundaceae are classified into three genera, *Osmunda*, *Leptopteris* and *Todea*, among which *Osmunda* are subdivided into subgenera *Osmunda*, *Osmundastrum* and *Plenasium* (sometimes raised to genera). By marked contrast to this, Yatabe *et al.*'s (1999) *rbcL* tree shows that *O. cinnamomea* is sister to all other members of the family including *O. claytoniana*, although the two species are usually assigned to subgenus *Osmundastrum*. Osmundaceae except for *O. cinnamomea* diverge into two clades. One comprises *Todea* and *Leptopteris* and the other comprises *O. claytoniana* and a subclade comprising a subgroup of *O. japonica*, *O. lancea* and *O. regalis* and subgenus *Plenasium*. This phylogeny suggests little morphological changes between *O. cinnamomea* and *O. claytoniana* since the earliest divergence of the extant Osmundaceae, as also shown by fossil evidence (Triassic *O. claytoniites*). Yatabe *et al.* (1999) estimated the divergence time to be 294 or 322 Mya for the two species, 210 Mya between the group of *Todea* and *Leptopteris* and the rest of the family, and 150 Mya between the *O. japonica* group and subgenus *Plenasium*.

Another divergence time estimate was made for extant lycophytes based on *rbcL* sequences (Wikström & Kenrick 2001). By calibration using several fossil evidence constraints Wikström & Kenrick (2001) estimated that the divergence time of the ligulate heterosporous group (*Isoetes*, *Selaginella*) and the nonligulate homosporous Lycopodiaceae is 393 Mya, that of *Isoetes* and *Selaginella* is 375 Mya, and that of *Huperzia* (also *Phylloglossum*) and a group of *Lycopodium* and *Lycopodiella* is 351 Mya. Data suggest that, in sharp contrast, the diversification of epiphytic species of *Huperzia*, like polypodioid ferns, occurred in the Late Cretaceous subsequently to the diversification of angiosperms.

Des Marais *et al.* (2003) performed a molecular analysis of *Equisetum*. They found that subgenera *Hippochaete* and *Equisetum* are each monophyletic, except for *E. bogotense* whose placement is ambiguous. Divergence time estimation shows that the modern *Equisetum* began divergence in the Early Cenozoic (Eocene; 40 Mya) and the two subgenera diverged in the Oligocene (30 Mya), *i.e.*, much later than the Paleozoic origin of Equisetaceae. The estimate is in accordance with fossil evidence. A distinct gap between the two divergence times indicates that cladogenesis seldom occurred or, more likely, extinction often happened during the period.

In conclusion, molecular divergence time estimates have demonstrated a historical structure of pteridophytes for over 400 million years. Estimation will become more accurate, as fossil and molecular

data will be added and calibration methods will be improved.

Cladistic Analyses

Pryer *et al.* (1995) undertook the first cladistic analysis of phylogenetic relationships and morphological evolution of ferns. They used 77 vegetative and reproductive characters for 50 taxa representing all major groups. Pryer *et al.* (1995) hypothesized an evolution of sporophytic and gametophytic characters, based on a phylogenetic tree constructed from combined morphological and molecular data. The characters examined include venation, hydathodes, dermal appendages, sporangial annulus and stalk, exospore, gametophyte hairs, antheridium position, and the number of antheridium wall cells.

Rothwell (1999) investigated the cladistic phylogeny of pteridophytes using morphological characters of both living and fossil plants, with special reference to ferns in a broad sense. Stauropterid ferns (fossils) are a monophyletic group that is basal in the pteridophytes and sister to the rest of the plants. Psilotaceae is next basal and most closely related to extinct primitive vascular plants. Cladoxylalean ferns plus Zygopteridalean ferns (both of which are fossils) form a clade that is more closely related to equisetophytes and seed plants than to other groups of fernlike plants. The basal filicalean ferns include living basal ferns and the Paleozoic ferns that are considered to be coenopterid ferns. Differences between Rothwell's (1999) and molecular phylogenies merit further analyses, because the former dealt with both living and extinct plants, while the latter dealt with living plants alone.

Kenrick & Crane (1997) carried out a large-scale cladistic analysis of early land plant evolution, based on evidence from both fossil and extant plants. According to them, polysporangiate protracheophytes with multiple sporangia on branched axes (*e.g.*, *Aglaophyton*, *Horneophyton*) preceded tracheophytes (Kenrick 2000). Kato & Akiyama (in press) interpreted that the branched axis of the polysporangiophyte with a persistent apical meristem is a novel vegetative organ interpolated into the bryophytic life cycle, and the bryophyte sporophyte is an archaic sporangium with the foot. The nonvascular polysporangiate plants had hydroid-like conducting cells without secondary walls. The vascular system increasingly developed as the axes enlarged. Early vascular plants had three different types of tracheids, S-type (*e.g., Senicaulis* of Rhyniopsida), G-type (*e.g., Gosslingia* of Zosterophyllopsida), and P-type (*e.g., Psilophyton* of Euphyllopsida) (Kenrick & Crane 1997). S-type tracheids have helical wall thickenings comprising a thin decay-resistant inner layer (facing the primary cell wall) and a spongy outer layer. G-type tracheids have annular thickenings comprising a decay-resistant inner layer and a nonresistant outer layer. P-type tracheids had scalariform thickenings comprising a decay-resistant inner layer and pit chambers, and a nonresistant layer. Fossils with G- and P-type tracheids are grouped in eutracheophytes, while Rhyniopsida with simple S-type tracheids is primitive tracheophytes placed outside the eutracheophytes. Friedman & Cook (2000) stressed the hypothetical evolution of developmental elaboration involving thickening of the decay-resistant layer, from S-type to G-type, then to P-type and eventually seed-plant type with no decay-prone layer.

Living eutracheophytes are divided into Lycophytina and Euphyllophytina. This biphyletic classification is in agreement with two branching patterns of roots in pteridophytes, *i.e.,* apical and dichotomous branching with exogenous origin in lycophytes and subapical or monopodial one with endogenous origin in ferns + *Equisetum* (Kato & Imaichi 1997). Zosterophyllopsida of Lycophytina has enations on axes and Lycopsida has microphyllous leaves, which are derived from enations. Leafy Euphyllophytina has euphyllous leaves and ancestral fossils had leafless branch systems or

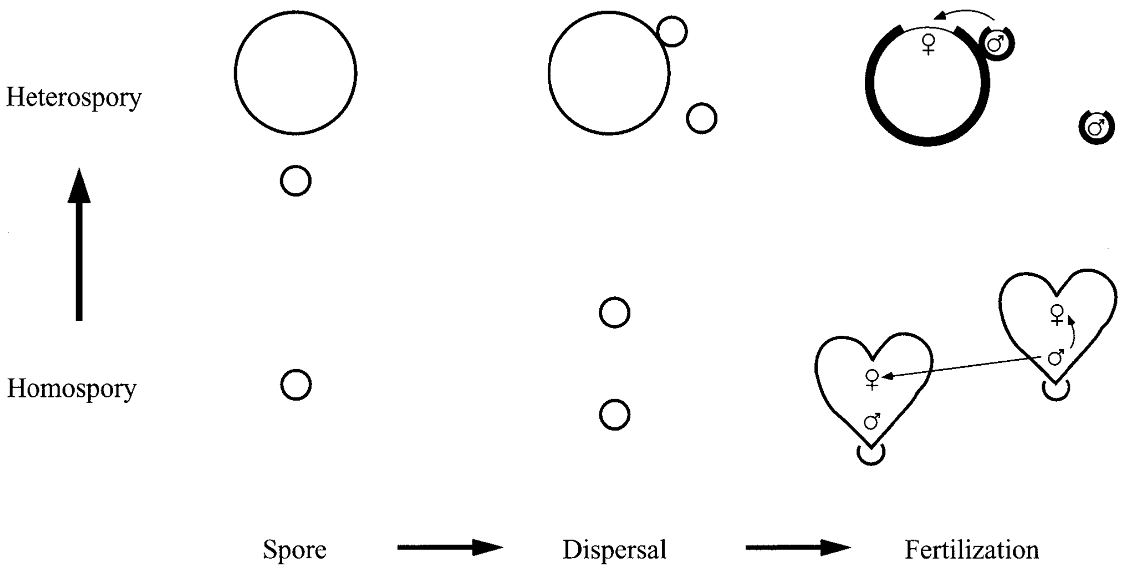

FIG. 2. Hypothetical spore dispersal and subsequent fertilization in heterosporous pteridophytes, compared with those of homosporous pteridophytes. In homosporous plants either intragametophytic selfing or intergametophytic crossing occurs in bisexual gametophytes derived from separately dispersed spores. Gametophytes are exosporic. In heterosporous plants co-dispersal of megaspores and microspores may occur by same migrating vector or spore pollination, and intergametophytic crossing occurs between connected or nearby unisexual gametophytes. Gametophytes are endosporic.

primitive leaves. Euphylls are considered to have been derived from telomes, *i.e.,* ancestral branched axes, but to have appeared independently in different lineages (at least ferns, progymnosperms, and equisetophytes). Beerling *et al.* (2001) and Osborne *et al.* (2004) suggested that changes of the Devonian atmospheric environment (CO_2 concentration, temperature) and the histological structure of leaves (stomata density, surface area, conducting system) forced the long (40 million years) delay of evolution of megaphylls behind leafless axes or microphylls.

Heterosporous Pteridophytes

Heterospory is considered to be an evolutionary prerequisite to a seed habit. A group of heterosporous progymnosperms evolved into seed plants, which have thrived in the Mesozoic and Cenozoic, most remarkably as angiosperms in the Tertiary up to the present. This clade of progymnosperms and seed plants is coined lignophytes characterized by the secondary vascular tissue. Heterospory appeared at minimum 10 times in most (5) of major lower vascular plant groups during the Middle Devonian and Permian (Bateman & DiMichele 1994, Bateman 1996). They are Lycopsida (Selaginellales [extant *Selaginella*] and Rhizomorphales [*Isoetes*]), Zosterophyllopsida (Barinophytales), Sphenopsida (some Sphenophyllales), Pteropsida (Stauropteridales), and Progymnospermopsida (some Aneurophytales, some Archaeopteridales, Protopityales, Cecropsidales, and some Noeggerathiales). In comparison, a few heterosporous ferns or Pteropsida (Salviniaceae [*Salvinia*, *Azolla*], Marsileaceae [*Marsilea*, *Pilularia*, *Regnellidium*], *Platyzoma*) evolved more recently, probably in the Jurassic or later (Pryer *et al.* 2004b). Thus, heterospory appeared iteratively throughout the history of pteridophytes. Heterospory did not evolve in early vascular plants such as Cooksoniopsida, Rhyniopsida, Trimerophytopsia and Cladoxylopsida that lived from the Late Silurian to the Early Carboniferous.

Heterospory is linked to gametophyte unisexuality: megapores exclusively produce female megagametophytes and microspores produce male

microgametophytes (Fig. 2). It results in obligate intergametophytic outcrossing, which maintains genetic variations that provide source of evolution to be selected. In this aspect, heterospory is more advantageous than homospory that allows intragametophytic selfing, although it is more disadvantageous in that dispersed spores, if single, cannot lead to fertilization and colonization in a new location except for apogamy. *Platyzoma* shows incipient heterospory (Tryon 1964). The megaspore is two times larger than the microspore, a small size difference compared to about 10 times larger megaspores of genuine heterosporous pteridophytes. The microspore germinates and develops into a small filamentous (exosporic), male gametophyte, while the megaspore develops into a large spatulate female gametophyte, which forms lobes with antheridia, eventually becoming hermaphroditic (Duckett & Pang 1984). Subcultured portions of those gametophytes originating from either spores yield male, female and hermaphroditic gametophytes at various proportions. Results indicate that the species has an association between gametophyte morphogenesis and sex organ formation, but the association is not absolute. In *Ceratopteris thalictroides* (Schedlbauer 1976) and other homosporous ferns, larger spores from a unimodal spore-size range tend to develop into bisexual gametophytes and the smaller into males, a similar sexual expression to that of *Platyzoma*. Duckett & Pang (1984) compared the sexual behavior of gametophytes of homosporous ferns with mixed and allopatric or allochronic gametangia, and suggested that such association of gametophyte dioecism and dimorphism evolutionarily may have preceded true heterospory, and sex determination may have been accelerated from the gametophyte stage to sporogenesis.

Endospory is exclusively associated with heterospory in living pteridophytes (Fig. 2). In endosporic species the gametophyte develops while a most portion is enclosed within the spore wall and precociously produces either antheridia or archegonia. After one or several cell divisions the microspore becomes a prothallial cell and (an) antheridium initial(s), and subsequently it (they) develop(s) into antheridia, which are embedded or protruded (Smith 1955). The megaspore undergoes cell divisions in the distal part and free nuclear divisions in the rest, and archegonia are formed in the cellular distal tissue. Thus, the male gametophyte is mostly antheridial, while the female gametophyte has food reserves that are also provided to the embryo. Sexual organ differentiation occurs at the initial stage of gametophyte development, and the female gametophyte bridges the sporogenesis and embryogenesis.

In heterosporous plants sexual determination is sporophytic and, unlike that of homosporous plants, is not influenced by the environment. DiMichele *et al.* (1989) and Bateman & DiMichele (1994) argued that the precociousness for endospory and gametophyte sexual maturation may possibly be a result of a kind of heterochrony (*i.e.,* progenesis). Earlier ideas of gradual evolution (*e.g.,* Tiffney 1981) claim that heterospory with exosporic unisexual gametophytes, as seen in *Platyzoma*, is an evolutionary intermediate between homospory (and exospory) and heterospory with endosporic gametophytes. Even though endospory followed heterospory, endospory may have occurred unnecessarily simultaneously in the megaspore and microspore. DiMichele *et al.* (1989) and Bateman & DiMichele (1994) do not support this gradual evolution hypothesis because of the disadvantage that free-living (exosporic) unisexual gametophytes cannot control the sex ratio and lose sexual flexibility (*e.g.,* lacking of intragametophytic selfing). DiMichele *et al.* (1989) and Bateman & DiMichele (1994) insist that heterospory is not a necessary antecedent to endospory, but rather may have evolved as an epigenetic consequence of endospory. They hypothesized that gametophytic unisexuality is a position effect of the metabolic microenvironment for developing spores: megaspores with female

gametophyte sexuality are produced at the tip of the soral receptacle that receives much nutrient supply, and microspores with male gametophyte sexuality are produced at the sides with less supply. This may be applicable for aquatic ferns (*Marsilea*, *Salvinia*, *Azolla*) with such a soral structure, but not obvious for *Selaginella* without comparable positional relationships. The evolution of heterospory and endospory (and also endosporangy related to the origin of the ovule) needs further analysis.

Heterospory is present in aquatic pteridophytes with an exception of *Selaginella*, which might be secondary terrestrial plants retaining air cavities around the vascular tissue. Heterosporous plants dominated tropical aquatic and amphibious habitats through most of the Carboniferous (DiMichele *et al.* 1985, 1992). They suggest that heterospory evolved in aquatic or semi-aquatic environments. Aquatic environments are favorable for the release of spermatozoids and eggs, which are produced from fast-developing, short-lived gametophytes, and for the consequent aquatic fertilization. These gametophytes exhibit a very shorter time lag from spore maturation to fertilization in, *e.g., Marsilea* than those of homosporous ferns (Schneider & Pryer 2002). Bateman & DiMichele (1994) regarded endosporic gametophytes as effectively functioning as gametes. In this context it cannot be ruled out that certain progymnosperms that evolved into seed plants had been aquatic or amphibious heterosporous pteridophytes. If it is the case, the early evolution of seed plants might have been accompanied with habitat transfer from aquatic to terrestrial environments.

Prior to fertilization, heterosporous pteridophytes have to undertake successful dispersal. Both megaspores and microspores must be dispersed within short distances to undergo intergametophytic fertilization. This situation may be achieved by chance or co-dispersal of both spores by means of the same vector (*e.g.,* birds) (Taylor *et al.* 1993) and/or physical connection of microspores on the megaspore surface before dispersal (Webster 1979, Takamiya 1999) (Fig. 2). This microspore-on-megaspore connection may be called spore pollination, compared to the pollen-on-stigma or micropyle pollination in seed plants. Co-dispersal of separate microspores and megaspores and/or spore-pollinated megaspores by the same vector is likely inevitable for oceanic island species that migrate by long-distance dispersal. There are some endemic heterosporous pteridophytes on oceanic islands, *e.g., Isoetes hawaiiensis*, *Marsilea villosa*, *Selaginella arbuscula*, and *S. deflexa* of the Hawaii (Palmer 2003) and *S. bonincola* (?) of the Bonin Islands (Iwatsuki *et al.* 1995). It is more probable that colonizers of these species were established by co-dispersal rather than by independent dispersal. Spore pollination may happen between different sporophytes or in the same sporophyte, and offspring resulting from spore self-pollination has as low genetic variability as that from self pollination. Limited co-dispersal of self-pollinated spores may promote isolation and speciation. Spore self-pollination may have a disadvantage with potential inbreeding depression.

This disadvantage might have been overcome by gymnosperms with dioecism in the lineage of lignophytes. Fossil data suggest that hydrasperman ovules or preovules with a salpinx (lagenostome) surrounded by a distally divided integument evolved into modern ovules with a micropyle formed by a cupulate integument (Rothwell & Scheckler 1988). The increasing efficiency of pollen capture by the complete integument, as shown by a wind-pollination experiment using preovule and ovule models and pseudopollen, is suggested to have been a driving force for fusion of integumentary lobes (Niklas 1981). Whether (pre)ovules facilitated cross pollination in early heterosporous gymnosperms is an interesting issue with reference to the evolution of seed plants from heterosporous pteridophytes (progymnsperms).

Pteridophyte Groups

Recent progress in molecular phylogeny (*e.g.*, Hasebe *et al.* 1995, Pryer *et al.* 2001, 2004b, Schneider *et al.* 2004a, b) has accompanied redefinition of pteridophytic groups and proposal of new groups (Fig. 1). Most of monophyletic and paraphyletic groups below are the same as those of Pryer *et al.* (2004b), and the phylogenetic relationships of monophyletic groups are shown with illustrations by Pryer *et al.* (2004a). Monophyletic groups should be given appropriate taxonomic ranks to demonstrate phylogenetic relationships. Paraphyletic groups are not usable as taxonomic units in a strict sense, but may be useful in non-taxonomic, general consideration, representing evolutionary stages.

Monophyletic groups

Polysporangiophytes — This group comprises nonvascular and vascular plants with branched aerial axes with multiple sporangia. Nonvascular members have bryophytic hydroid- and leptoid-like conducting cells and are known as fossils alone (*e.g., Aglaophyton*, *Hornophyton*). Polysporangiate plants are sister to nonvascular bryophytes with unbranched, monosporangiate sporophytes. For the evolution of polysporangiate branched axes see Kato & Akiyama (in press).

Tracheophytes (*vascular plants*) — This group is defined by having vascular tissues and comprises euphyllophytes and microphyllous lycophytes. It also includes part of leafless "Rhyniopsida" (*e.g., Cooksonia*) (Rothwell 1999, Pryer *et al.* 2004b). Tracheophytes are a member of polysporangiophytes and may be divided into eutracheophytes and primitive tracheophytes.

Lycophytes (*microphyllophytes*) — This group is characterized by the microphyllous leaves with single sporangia on the adaxial side of leaves or in the axil. It is sister to the euphyllophytes. The ancestral zosterophytes with nonleaf enations have sporangia lateral on the axes. Extant lycophytes include the homosporous eligulate Lycopodiaceae and the heterosporous ligulate Isoetaceae and Selaginellaceae.

Euphyllophytes — This group is characterized by the euphyllous or non-microphyllous leaves and comprises monilophytes and seed plants. Euphylls (true leaves) include fern megaphylls, seed plant megaphylls, enation-like or ensiform leaves of Psilotaceae, and sphenophylls of Equisetaceae, although they may have evolved recurrently in different lineages. Euphyllophytes = tracheophytes − lycophytes.

Monilophytes — This group comprises three free-sporing groups, *i.e.,* ferns (which are not monophyletic), Psilotaceae and Equisetaceae, the last two of which are conventionally separated as fern allies. The monilophytes are sister to seed plants and defined by the euphyllous leaves (megaphylls or sphenophylls [in *Psilotum* simple or forked enation-like], *i.e.,* non-microphyllous leaves) and absence of secondary vascular tissue in addition to pteridophytic reproduction. Monilophytes = euphyllophytes − spermatophytes.

Leptosporangiate ferns — Filicalean ferns with leptosporangia that develop from single surface cells of megaphyllous leaves. Osmundaceae, which diverge the earliest among the leptosporangiate ferns, show variable sporangium developmental patterns, part of which is similar to the eusporangial pattern with a square-based archesporial cell, and the sporangia are massive and produce 128-512 spores, an output intermediate between 1000 or more in a eusporangium and typically 64 in a leptosporangium (Bower 1935).

Aquatic ferns (*hydropteroids*) — This group comprising Marsileaceae and Salviniaceae (usually including Azollaceae) is defined by the heterospory and aquatic life form (Fig. 1A), although the plants vary so remarkably as to be sometimes classified at the order or higher rank. Phylogenetically the aquatic ferns are not close to the heterosporous amphibi-

ous leptosporangiate fern *Platyzoma* (Pteridaceae), and to the homosporous aquatic fern *Ceratopteris*, and are very remote from the heterosporous aquatic *Isoetes* of the microphyllous lycopods.

Polygrammoid ferns—These ferns comprise Polypodiaceae and Grammitidaceae, hence the name is derived from a combination of the family names (Schneider *et al.* 2002). Grammitidaceae are sister to *Polypodium* (*P. triseriale* group) within Polypodiaceae (Fig. 1B). The polygrammoids are epiphytes with usually densely scaly, long-creeping rhizomes and exindusiate discrete (round or elongate) sori superficial on the leaf surface.

Lignophytes—This group comprises spermatophytes (seed plants) and pteridophytic progymnosperms that are a free-sporing immediate ancestor. The shared wood or secondary vascular tissue is produced by the bifacial cambium and supports arborescence, which is advantageous for light capture. It is likely that the progymnosperms ancestral to seed plants were heterosporous, although DiMichele *et al.* (1989) assumed that they were homosporous.

Paraphyletic groups

Pteridophytes—These plants are free-sporing (non-seed) vascular plants comprising ferns, three fern allies (see below). Among them, monilophytes (ferns, Psilotaceae and Equisetaceae) are sister to non-pteridophytic seed plants and together sister to another ally, lycophytes.

Ferns—This group with circinate, spore-bearing megaphyllous leaves include eusporangiate and leptosporangiate ferns. The ferns have conventionally been treated as a single taxonomic group. However, the eusporangiate Marattiaceae and Ophioglossaceae form monophyletic groups along with the fern allies Equisetaceae and Psilotaceae, respectively.

Fern allies—These share free-spore reproduction with ferns. Three fern allies, lycophytes, Psilotacae and Equisetaceae, are often treated at the same class level as ferns. Different from megaphyllous ferns, they have non-megaphyllous leaves (microphylls in lycophytes, sphenophylls in Equisetaceae, and simple or forked enation-like or ensiform leaves in Psilotaceae). The paraphyly of the fern allies is shown by the phylogeny in which the lycophytes are sister to the rest of vascular plants, while Psilotaceae and Equisetaceae are assigned to the monilophytes.

Eusporangiate ferns—Megaphyllous ferns with eusporangia that develop from multiple initial cells. Marattiaceae and Ophioglossaceae are such extant ferns. The eusporangia are plesiomorphic, possibly derived from those of the ancestral bryophytes, and shared by Psilotaceae, Equisetaceae, seed plants, and lycophytes, *i.e.,* all vascular plants except leptosporangiate ferns. The last alone have derivative leptosporangia.

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